

Supplementary information

Vaccinia virus hijacks EGFR signalling to enhance virus spread through rapid and directed infected cell motility

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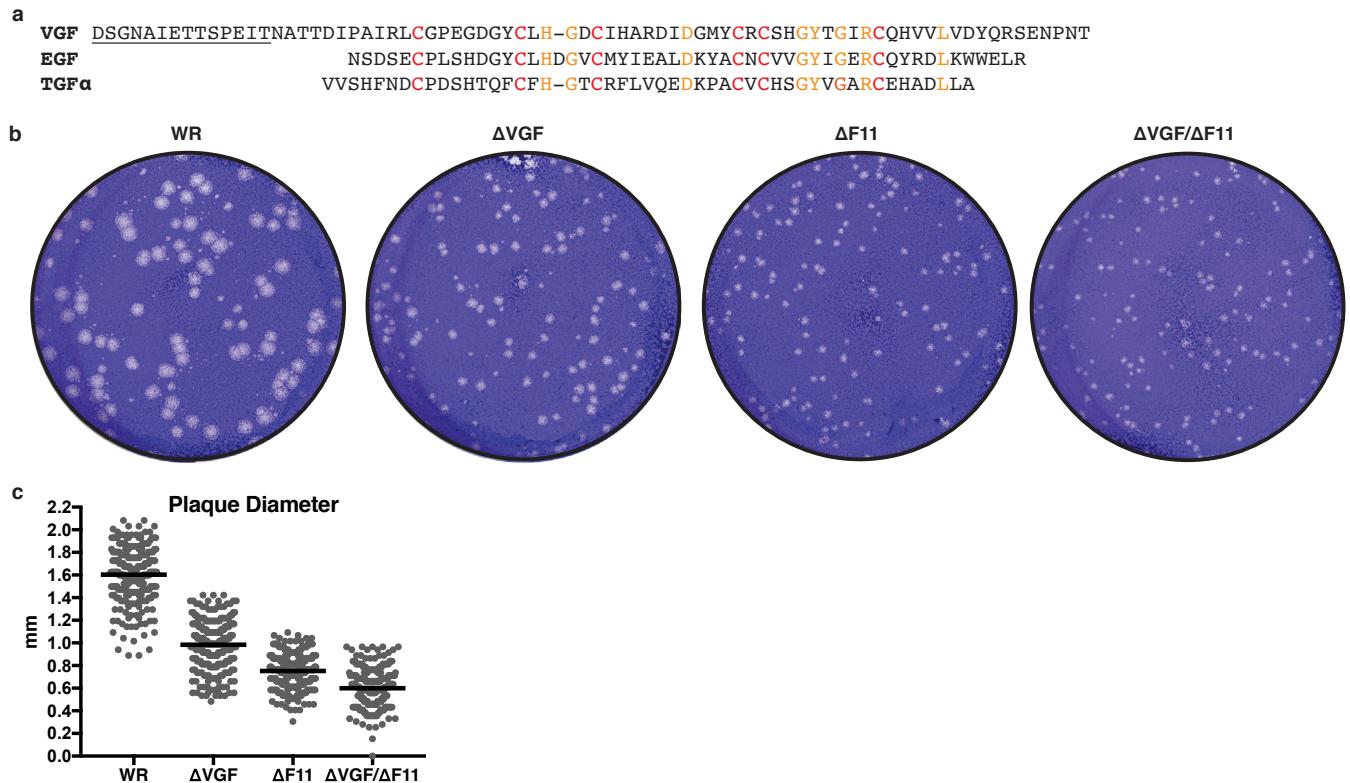
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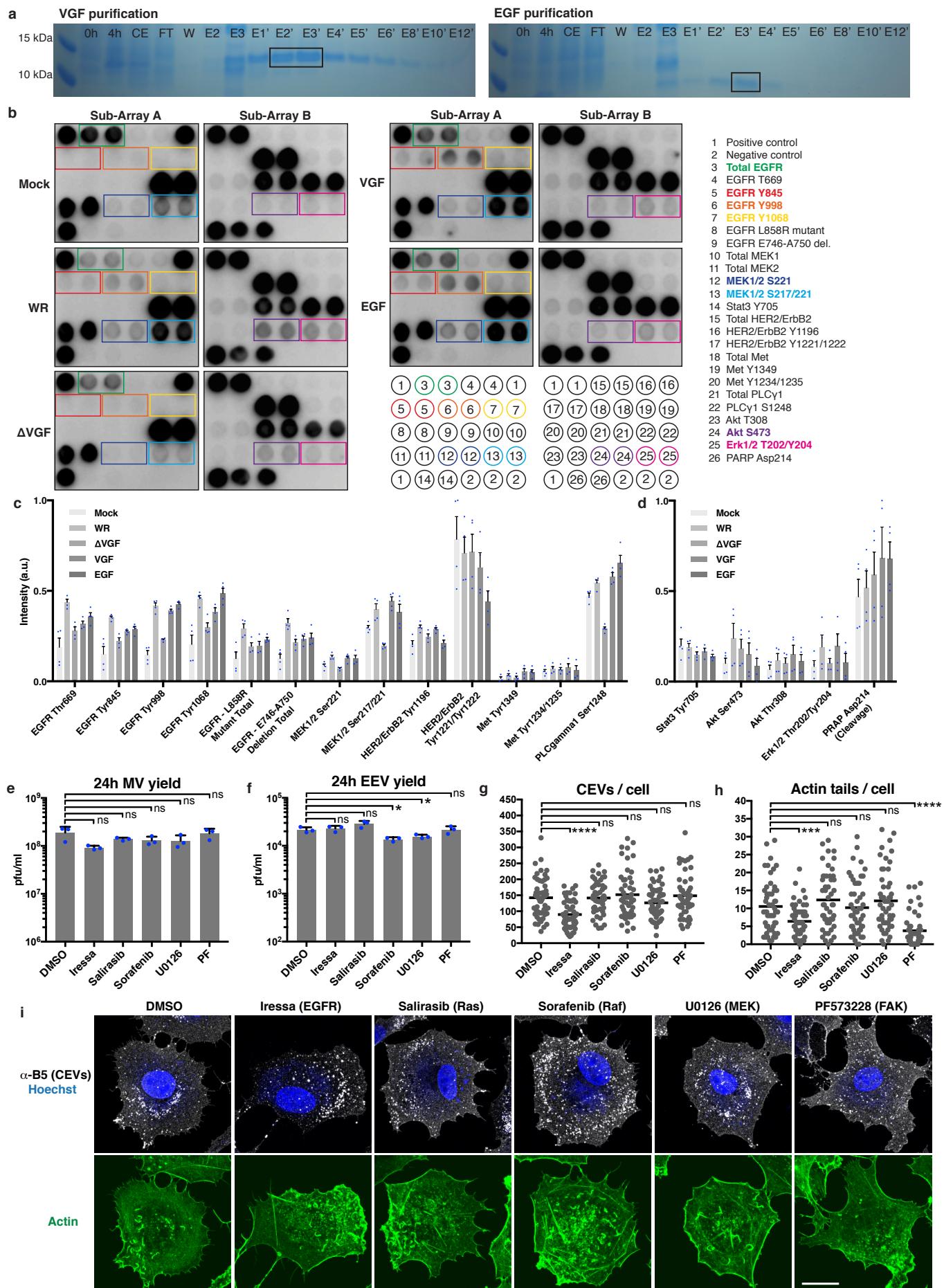
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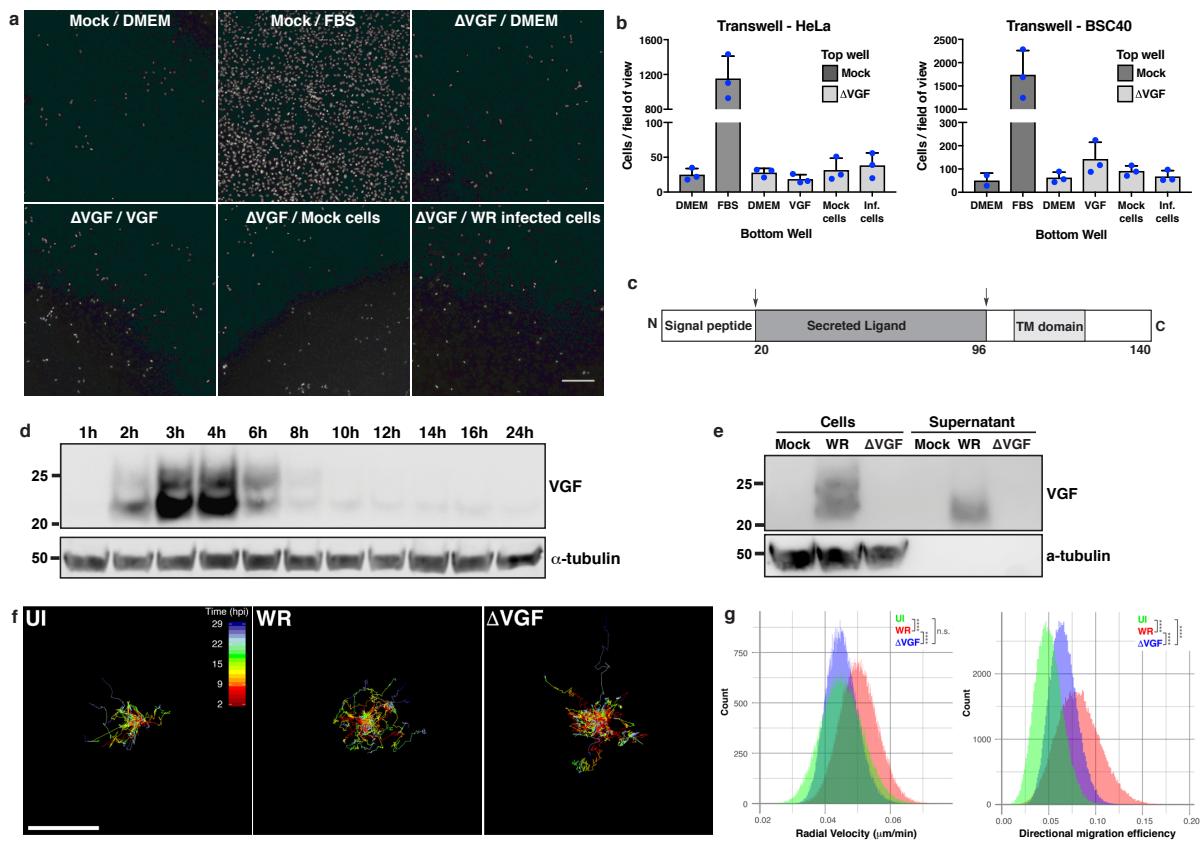
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Supplementary Figure 1. VGF is an EGF homolog required for VACV plaque formation. **a**, Alignment of secreted VGF with human EGF and TGF α . Homologous amino acid sequences are highlighted. The amino acid sequence used to produce α -VGF antibody is underlined. Adapted from¹⁴. **b**, Comparison of WR, Δ VGF, Δ F11 and Δ VGF/ Δ F11 plaque size. Plaques visualized by crystal violet staining at 48 hpi. **c**, Quantification of plaque diameters. Representative data from 3 biological replicates (**b**, **c**). Lines represent mean of 100 plaques per condition/replicate (**c**). See Supplementary Table 1 for exact statistics.

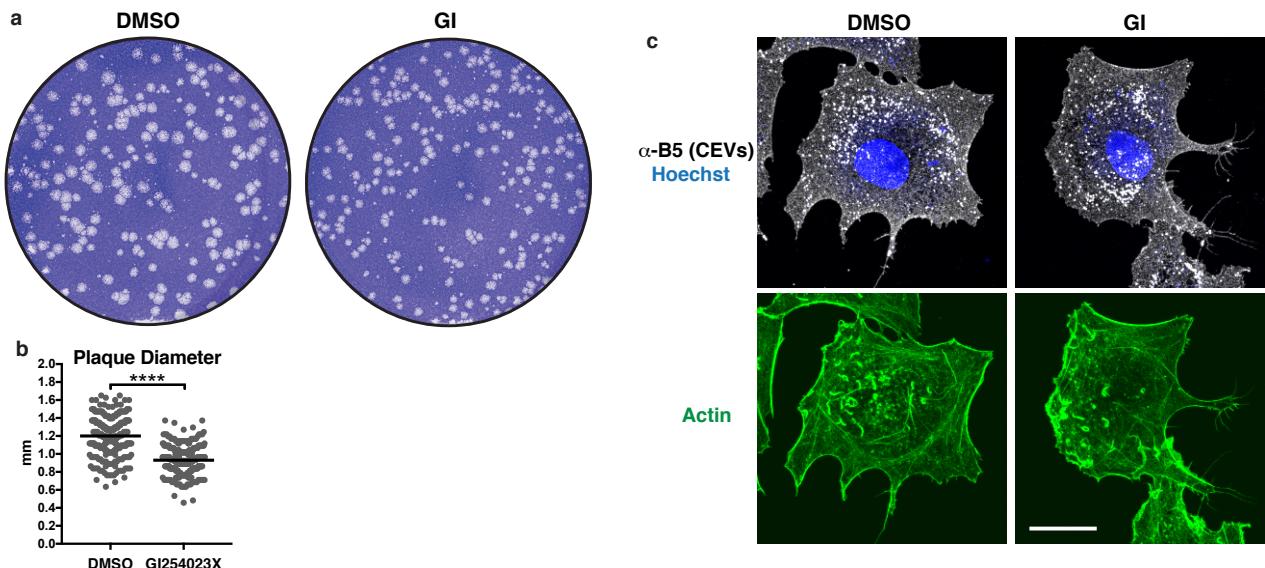


Supplementary Figure 2. VGF activates EGFR/MEK/ERK signalling. **a**, Purification of His-tagged VGF and His-tagged EGF. E1-E3: Elution with 125 mM imidazole. E1'-E12': Elution with 250 mM imidazole. Fractions used for experiments are boxed. **b**, An EGFR PathScan activation array was performed on lysates from HeLa cells infected with WR, Δ VGF or treated with recombinant VGF or EGF. Proteins that undergo changes in phosphorylation under these conditions are boxed. **c, d**, Quantification of EGFR arrays with (**c**) or without (**d**) normalisation to total protein. **e, f**, 24 h MV and EEV yields from inhibitor-treated WR infected cells. **g, h**, CEVs and actin tails per cell during WR infections in the presence of indicated inhibitors for 10 h. **i**, Representative images of inhibitor-treated cells infected for 10 h. Scale bar = 20 μ m. Representative data from 2 (**b-d**) or 3 (**e-i**) biological replicates. Bars represent means + SEM (**c, d**) or means + SD (**e, f**). Lines represent means of 15-20 cells per replicate (**g, h**). Paired (**e, f**) or unpaired (**g, h**) t-test was applied (**** P<0.0001, *** P<0.001, * P<0.05, ns = not significant). See Supplementary Table 1 for exact statistics.



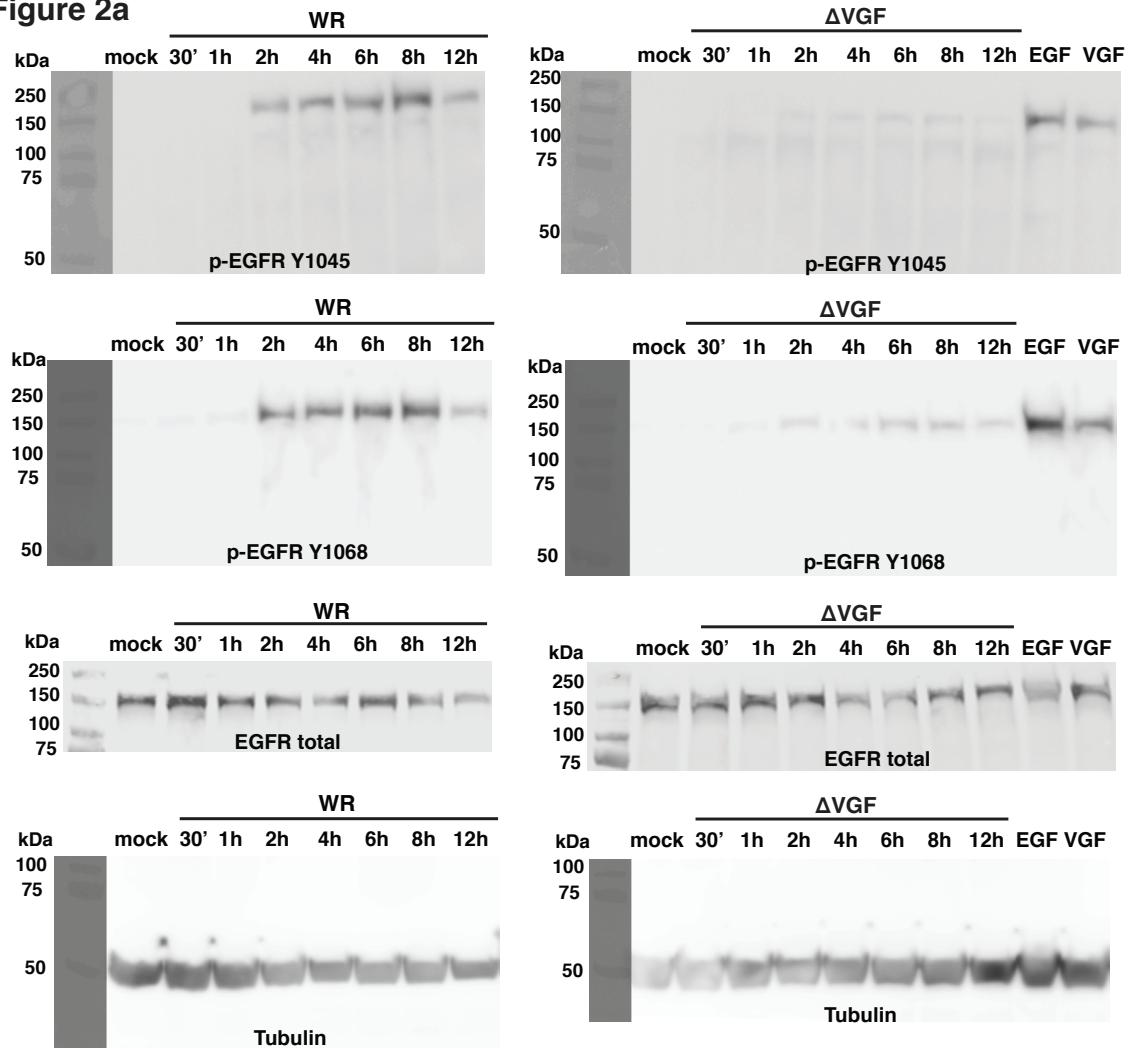
Supplementary Figure 3. VGF is secreted early, is not a chemoattractant and displays moderate autocrine activity. **a**, Representative images of the bottom side of transwell membranes. Nuclei of BSC40 cells were stained with Hoechst. **b**, Quantification of BSC40 and HeLa cell migration in transwell assays. **c**, Schematic of VGF precursor protein. Arrows indicate sites of cleavage. Adapted from¹³. **d**, Time course of VGF expression in WR infected cells from 1 to 24 hpi. **e**, Secretion of VGF from WR or Δ VGF infected cells. Cells and concentrated supernatants were analysed by anti-VGF immunoblot. **f**, Tracks of individual uninfected (UI), WR or Δ VGF infected cells normalized from the coordinates of the point of origin and superimposed. Tracks are colour-coded by time. Autocrine signalling in single infected (WR or Δ VGF) or uninfected cells (UI). **g**, Histograms of radial velocity ($\mu\text{m}/\text{min}$) and directional migration efficiency of single cell movement in **f**. Scale bars=200 μm . **(a-f)**. Representative data from 3 biological replicates **(a-e)**. Bars represent means + SD **(b)**. Thirty

individual cells/condition in biological replicates (**f**, **g**). See Supplementary Table 1 for exact statistics.

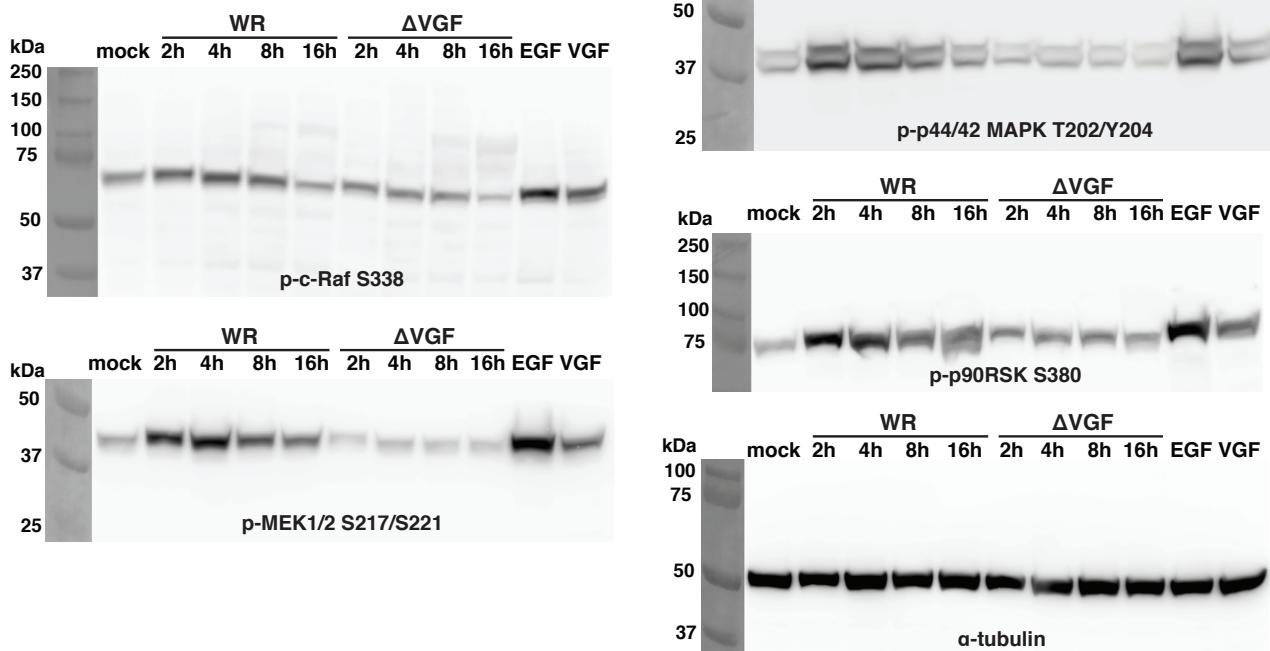


Supplementary Figure 4. Inhibition of ADAM10 reduces VACV plaque size without impeding CEV or actin tail formation. **a**, Comparison of WR plaque sizes in the presence of GI or DMSO control. Plaques were visualized by crystal violet staining at 48 hpi. **b**, Plaque diameters measured from **(a)**. **c**, Representative images of WR infected cells in the presence of DMSO or GI at 10 hpi. Representative data from 3 biological replicates **(a-c)**. Lines represent means of 100 plaques/condition/replicate **(b)**. Unpaired t-test was applied (***) $P < 0.0001$. See Supplementary Table 1 for exact statistics.

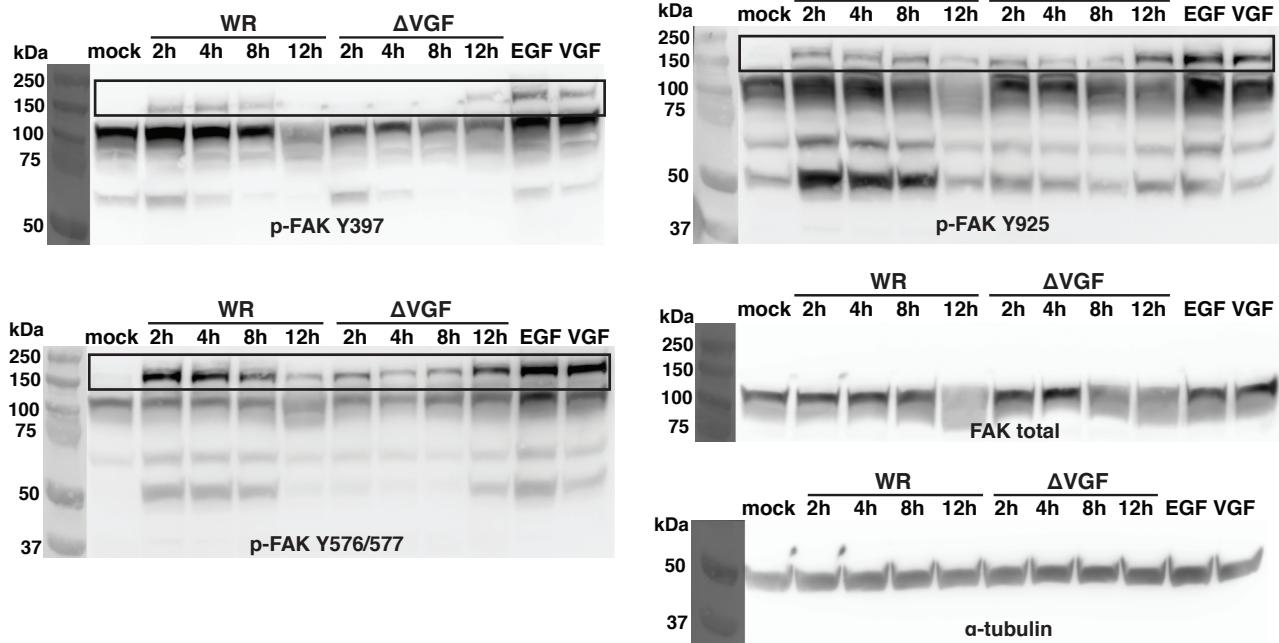
Blots Figure 2a



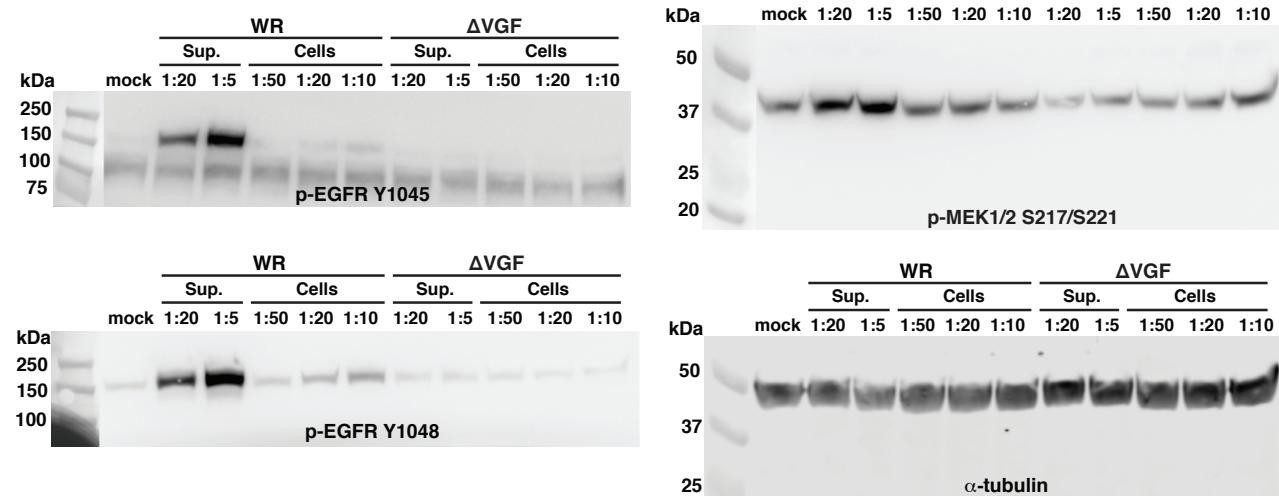
Blots Figure 2b



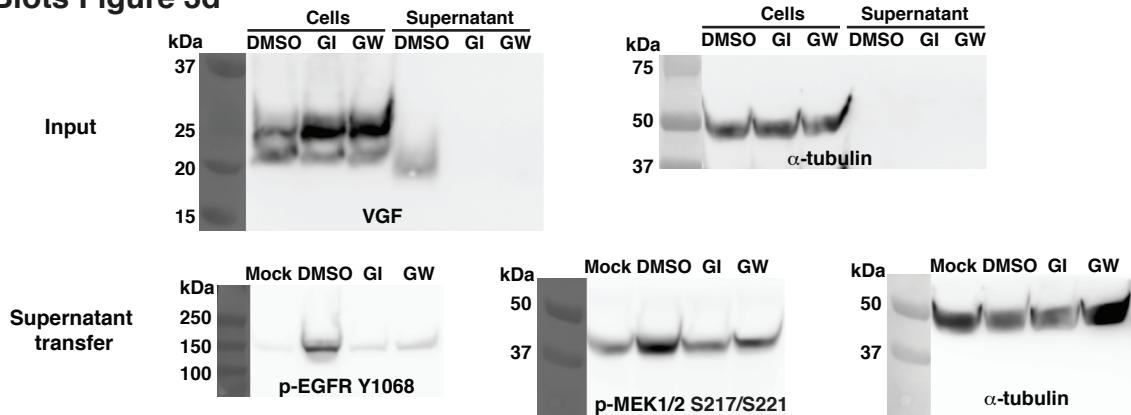
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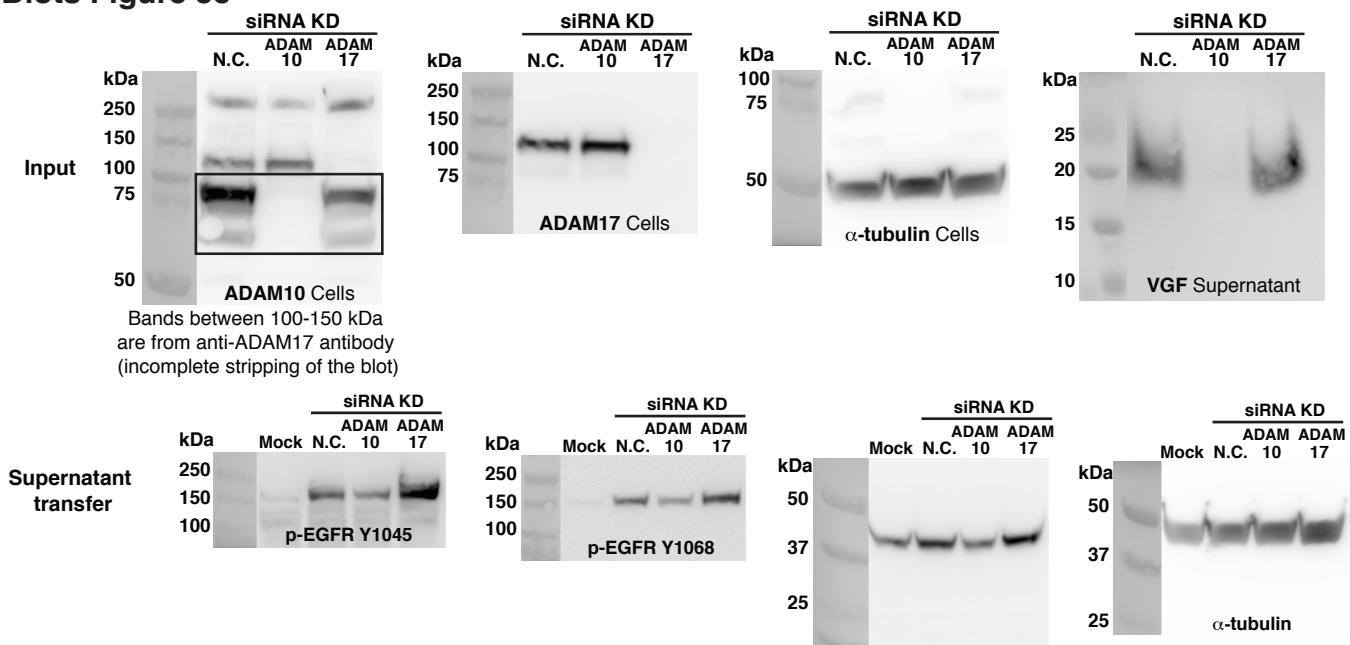
Blots Figure 3c



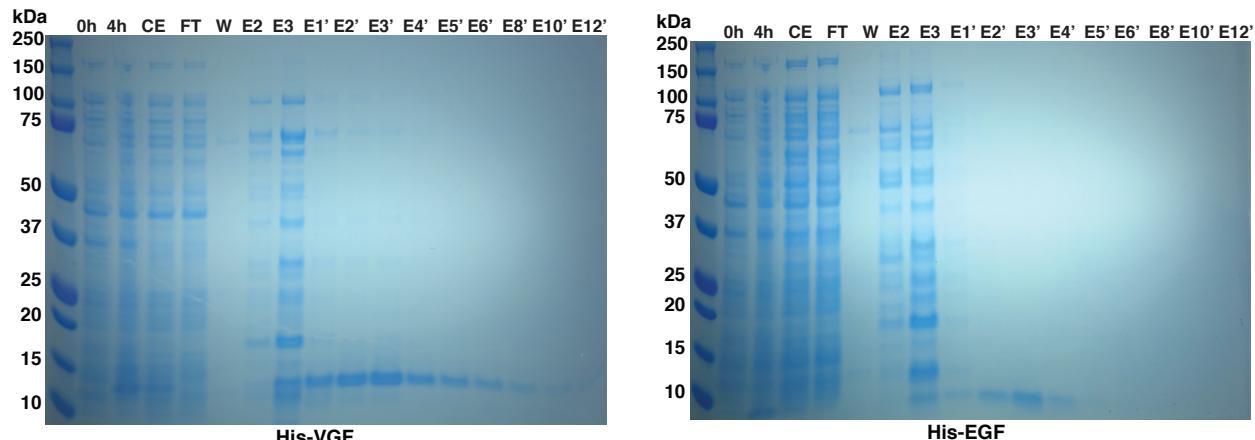
Blots Figure 3d



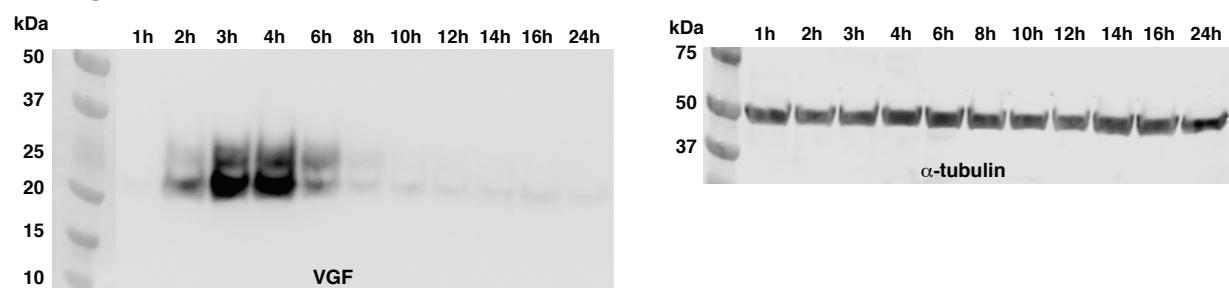
Blots Figure 3e



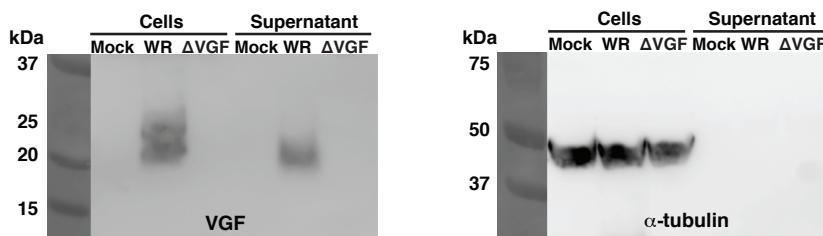
Gels Figure S2a



Blots Figure S3d



Blots Figure S3e



Supplementary Figure 5. Full layout of blots presented in main and supplementary figures. Uncropped images of the cropped blots and gels presented in Figures 2a to S3e.

Supplementary Table 1. Statistics and reproducibility. All statistical tests, biological replicates, exact p-values, and significance for all graphs in this manuscript.

Figure	Test	Comparison	P value	Significance
1b	Paired t-test, two-sided n=4 biologically independent experiments	WR vs. ΔVGF WR vs. ΔF11 WR vs. ΔVGF/ΔF11	0.001 0.9162 <0.0001	** ns ****
1c	Paired t-test, two-sided n=4 biologically independent experiments	WR vs. ΔVGF WR vs. ΔF11 WR vs. ΔVGF/ΔF11	0.5056 0.0378 0.3828	ns * ns
1d	Unpaired t-test, two-sided n=3 biologically independent experiments 17 cells per experiment/sample	WR vs. ΔVGF WR vs. ΔF11 WR vs. ΔVGF/ΔF11	0.6577 0.0409 0.9342	ns * ns
1e	Unpaired t-test, two-sided n=3 biologically independent experiments 17 cells per experiment/sample	WR vs. ΔVGF WR vs. ΔF11 WR vs. ΔVGF/ΔF11	0.248 0.8673 0.0149	ns ns *
1h	Unpaired t-test, two-sided n=3 biologically independent experiments 5 plaques per experiment/sample	WR vs. ΔVGF WR vs. ΔF11 WR vs. ΔVGF/ΔF11	<0.0001 <0.0001 <0.0001	**** **** ****
1i	Unpaired t-test, two-sided n=3 biologically independent experiments 5 plaques per experiment/sample	WR vs. ΔVGF WR vs. ΔF11 WR vs. ΔVGF/ΔF11	<0.0001 <0.0001 <0.0001	**** **** ****
2e	Unpaired t-test, two-sided n=3 biologically independent experiments 100 plaques per experiment/sample	DMSO vs. Iressa DMSO vs. Salirasib DMSO vs. Sorafenib DMSO vs. U0126 DMSO vs. PF573228	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001	**** **** **** **** ****
2g	Unpaired t-test, two-sided n=3 biologically independent experiments 5 plaques per experiment/sample	DMSO vs. Iressa DMSO vs. U0126 DMSO vs. PF573228	<0.0001 <0.0001 <0.0001	**** **** ****
2h	Unpaired t-test, two-sided n=3 biologically independent experiments 5 plaques per experiment/sample	DMSO vs. Iressa DMSO vs. U0126 DMSO vs. PF573228	<0.0001 <0.0001 <0.0001	**** **** ****
3g	Unpaired t-test, two-sided n=3 biologically independent experiments 5 plaques per experiment/sample	DMSO vs. GI	<0.0001	****
3h	Unpaired t-test, two-sided n=3 biologically independent experiments 5 plaques per experiment/sample	DMSO vs. GI	<0.0001	****
3i	Paired t-test, two-sided n=4 biologically independent experiments	DMSO vs. GI	0.6974	ns
3j	Paired t-test, two-sided n=4 biologically independent experiments	DMSO vs. GI	0.4624	ns
3k	Unpaired t-test, two-sided n=3 biologically independent experiments 17 cells per experiment/sample	DMSO vs. GI	0.4297	ns
3l	Unpaired t-test, two-sided n=3 biologically independent experiments 17 cells per experiment/sample	DMSO vs. GI	0.0703	ns

4c	Unpaired t-test, two-sided n= 2 mice per virus 12 lesions for WR, 10 lesions for Δ VGF	WT vs. Δ VGF	0.0016	**
4d	Unpaired t-test, two-sided n= 3 mice per virus 15 lesions/virus	WT vs. Δ VGF	<0.0001	****
S1c	Unpaired t-test, two-sided n=3 biologically independent experiments 100 plaques per experiment/sample	WR vs. Δ VGF WR vs. Δ F11 WR vs. Δ VGF/ Δ F11	<0.0001 <0.0001 <0.0001	**** **** ****
S2e	Paired t-test, two-sided n=3 biologically independent experiments	DMSO vs. Iressa DMSO vs. Salirasib DMSO vs. Sorafenib DMSO vs. U0126 DMSO vs. PF573228	0.1322 0.3226 0.1613 0.1325 0.9036	ns ns ns ns ns
S2f	Paired t-test, two-sided n=3 biologically independent experiments	DMSO vs. Iressa DMSO vs. Salirasib DMSO vs. Sorafenib DMSO vs. U0126 DMSO vs. PF573228	0.5556 0.0956 0.04 0.0328 0.9181	ns ns * * ns
S2g	Unpaired t-test, two-sided n=3 biologically independent experiments 17 cells per experiment/sample	DMSO vs. Iressa DMSO vs. Salirasib DMSO vs. Sorafenib DMSO vs. U0126 DMSO vs. PF573228	<0.0001 0.9703 0.4738 0.1474 0.6042	**** ns ns ns ns
S2h	Unpaired t-test, two-sided n=3 biologically independent experiments 17 cells per experiment/sample	DMSO vs. Iressa DMSO vs. Salirasib DMSO vs. Sorafenib DMSO vs. U0126 DMSO vs. PF573228	0.0006 0.2263 0.8282 0.2905 <0.0001	*** ns ns ns ****
S3g	Non-parametric resampling test (10 000 permutations), one sided	Uninfected vs. WR	<0.0001	****
Velocity	n=2 biologically independent experiments per condition 33 tracks for WR, 49 tracks for Δ VGF, 24 tracks for uninfected	Uninfected vs. Δ VGF WR vs. Δ VGF	0.9981 <0.0001	ns ****
S3g	Non-parametric resampling test (10 000 permutations), one sided	Uninfected vs. WR	<0.0001	****
Efficiency	n=2 biologically independent experiments per condition 33 tracks for WR, 49 tracks for Δ VGF, 24 tracks for uninfected	Uninfected vs. Δ VGF WR vs. Δ VGF	<0.0001 <0.0001	**** ****
S4b	Unpaired t-test, two-sided n=3 biologically independent experiments 100 plaques per experiment/sample	DMSO vs. GI	<0.0001	****